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Increased low density lipoprotein oxidation in stable kidney transplant recipients

HOSAM GHANEM, MARINUS A. VAN DEN DORPEL, WILLEM WEIMAR, ARIE J. MAN IN 'T VELD, MOHAMMED H. EL-KANNISHY and HANS JANSEN

Departments of Internal Medicine I and III and Biochemistry, Erasmus University Rotterdam, Rotterdam, The Netherlands

Increased low density lipoprotein oxidation in stable kidney transplant recipients. We studied factors that may add to the high risk of atherosclerosis in kidney transplant recipients. Plasma lipoprotein concentrations and parameters of low density lipoprotein (LDL) oxidation were determined in 19 clinically stable kidney recipients and 19 healthy controls. Plasma triglycerides and total cholesterol were increased in the patients. High density lipoprotein-cholesterol (HDL-c) was in the normal range. The mean LDL diameter was smaller in patients than in controls (236.5 ± 7.3 Å vs. 247.8 ± 11.6 Å, $P < 0.002$), which was due to a higher frequency of the LDL subclass pattern B in the patients than in controls (58% vs. 28%). The lag time of copper-induced *in vitro* LDL oxidation was shorter in patients than in controls (101 ± 23 min vs. 148 ± 81 min, $P = 0.02$). The titer and concentration of autoantibodies against malondialdehyde-modified (MDA-LDL) determined by ELISA were higher in the patients than in the controls. This difference was found in both IgG (titer + 9%, concentration + 75%; $P < 0.05$) and IgM (titer + 35%, concentration + 102%; $P < 0.001$). Based on these results, we propose that there is *in vitro* and *in vivo* evidence of enhanced LDL oxidation in patients post-renal transplantation. This might represent one cause for the clinical finding of advanced atherosclerosis in these patients.

Cardiovascular atherosclerosis is frequently found in patients with chronic renal insufficiency. If atherosclerosis is present before transplantation, it continues to progress after renal transplantation [1]. Several factors may contribute to the progression of the atherosclerosis [2]. Before transplantation, most patients are suffering from chronic renal failure, which is associated with a high incidence of atherosclerosis [3, 4]. After transplantation, there is an increased prevalence of the well-established risk factors, hypertension [5, 6] and hyperlipidemia [7–9]. Besides increased plasma lipoprotein concentrations, alterations in the composition [10] and susceptibility for oxidation [11] of the lipoproteins may also play a role in the atherosclerosis in kidney transplantation patients. Chemical modification of LDL, including oxidation, probably precedes the uptake of LDL by macrophages and the accumulation of cholesterol in the arterial wall [12, 13]. The presence of oxidatively modified LDL in atherosclerotic lesions supports this hypothesis [14–16]. The susceptibility of LDL for oxidation can be determined *in vitro* [17]. As a measure of the susceptibility of LDL for oxidation, the time that elapses before lipid peroxidation products become detectable (lag phase) can be measured [17]. The susceptibility of LDL for oxidation may be one factor determining LDL oxidation *in vivo*. The lag phase was found to be correlated with the extent of coronary atherosclerosis in humans [18]. Human plasma contains autoantibodies against epitopes of oxidized LDL [19, 20]. The level of these antibodies may also reflect the rate of LDL oxidation [19]. Progression of peripheral atherosclerosis correlates with the titer of these antibodies [20].

The lipoproteins in the LDL density range form a heterogeneous population of different sized particles [21–24]. LDL may be separated by size via gradient gel acrylamide electrophoresis; several LDL subclasses are distinguished in this manner [22]. If the LDL fraction contains mainly large LDL, this is designated as the LDL subclass pattern A. The presence of mainly small-dense LDL is indicated as the LDL subclass pattern B [25, 26]. The pattern B is associated with a high plasma triglycerides and a low HDL cholesterol concentration, and is partly determined by genetic factors [25, 26]. Subjects with the LDL subclass pattern B have an increased risk of coronary heart disease [25–29]. The atherogenicity of small-dense LDL may be due to its association with increased plasma triglycerides and lowered HDL cholesterol. On the other hand, small LDL may play a role in the onset or progression of CHD due to the physical and physiological properties of this LDL. Small LDL has a different carbohydrate composition than large LDL [30]. This may lead to an enhanced uptake of small LDL by intima-media [31]. In addition, small LDL is more prone to oxidative modification than larger LDL [32, 33]. Therefore, the LDL subclass pattern, the susceptibility of LDL for oxidation *in vitro*, and the level of autoantibodies to epitopes of oxidized LDL seem to be indicators of *in vivo* LDL oxidation. Besides the LDL cholesterol concentration, these variables may reflect the atherogenicity of the LDL fraction.

In this study, we determined these variables in renal transplantation recipients and matched controls.

Methods

Study population

We studied 19 stable kidney transplant recipients (13 males, 6 females) and 19 controls, matched for sex, age, and body mass index. The patients were considered to be in a stable condition if no rejection episodes or increase in serum creatinine of more than

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Table 1. Characteristics of patient and control groups

	Patients	Controls
Males *N*	13	13
Females *N*	6	6
Age *years*	42.2 ± 12.3	47.3 ± 9.1
Body mass index kg/m^2	24.9 ± 3.5	25.6 ± 3.1
Time after transplantation *m*	24 ± 5	—
Cyclosporin dose *mg/kg/day*	5.5 ± 1.7	—
Prednisone dose *mg/day*	9.6 ± 2.1	—
Serum creatinine *μM*	155 ± 44	76 ± 9
Creatinine clearance *ml/min*	59 ± 22	116 ± 29
Systolic blood pressure *mm Hg*	149 ± 20	—
Diastolic blood pressure *mm Hg*	97 ± 14	—

20 μmol/liter had occurred in the six months before blood sampling. None of the patients were treated with high doses of corticosteroids during this period. Exclusion criteria were the presence of proteinuria or diabetes. Before transplantation, one patient had been suffering of angina pectoris and one patient of intracerebral hematoma. The other patients had no cardiovascular problems before transplantation. At the time of blood sampling none of the patients showed signs of cardiovascular disease except hypertension. Nine patients were treated with calcium antagonists, nine with labetalol and one with metoprolol. All antihypertensive drugs were discontinued for at least three days before blood sampling.

None of the control subjects had overt atherosclerosis or used antihypertensive drugs. Table 1 shows demographic data of patients and controls.

Blood sampling

Blood samples were drawn into EDTA containing polypropylene tubes (final concentration EDTA, 1 mg/ml) on ice. Plasma was obtained by low speed centrifugation at 4°C. To the plasma sucrose (10 μl sucrose solution/ml plasma) was added to a final concentration of 0.6% to prevent LDL aggregation [34]. Plasma was stored at −80°C until use.

Separation of plasma lipoproteins

Low density lipoproteins for oxidation experiments were isolated by density gradient ultracentrifugation as described by Redgrave, Roberts and West [35]. To prevent oxidation of the lipoproteins during ultracentrifugation 0.1 mM EDTA and 0.005% thiomersal were added to the gradient solutions. Before use, the solutions were gassed by nitrogen to remove the oxygen. All runs were for 24 hours at 15°C and 40,000 rpm in SW 41 TI Beckman rotor using polyallomere tubes. HDL cholesterol was determined after precipitation of apoB containing lipoproteins with heparin/$MnCl_2$ [36]. The plasma LDL cholesterol concentration was calculated using the Friedewald formula [37].

Low density lipoprotein oxidation

Immediately after isolation part (300 μl) of the LDL fraction was placed in a microdialysis apparatus. Six samples were simultaneously dialyzed against 120 ml phosphate buffered saline (PBS, 0.15 M NaCl, 0.01 M phosphate, pH 7.4) for 48 hours at 4°C in the dark. The dialysis buffer was refreshed after 24 hours. The LDL cholesterol content was measured and the samples were diluted with PBS to a final concentration of 0.25 μM LDL-cholesterol. The LDL oxidation experiments were carried out as described by Esterbauer et al [17]. Six samples of LDL were measured simultaneously in a thermostated Perkin-Elmer Lambda 5 spectrophotometer at 25°C. Oxidation was initiated by the addition of a freshly prepared copper chloride solution (final concentration 1.66 μM). LDL oxidation was followed by monitoring the change in absorbance at 234 nm every two minutes for 16 hours. The lag time was defined as the interval between initiation of the reaction and the intercept of the tangent of the slope of the absorbance curve with the time scale axis expressed in minutes.

LDL size and subclass determination

The LDL subclass patterns were identified by electrophoresis on 2 to 16% PAGE gels, as described by Austin et al [25]. The gels were prepared with an LKB 11300 Ultrograd gradient mixer [38].

In each gel, reference sera with known subclass pattern were applied to lane one and six of a total 12 lanes. The gels were stained with Oil Red O for lipid and the subclass pattern determination.

For the determination of the LDL size, a set of standard proteins with known hydrated diameters was run on the same gel as the samples. The standard proteins (HMW electrophoresis calibration kit; Pharmacia, Piscataway, NJ, USA) were thyroglobulin (170 Å), ferritin (122 Å) and catalase (104 Å). The gels were stained with Coomassie Brilliant Blue R 250. The center of the most prominent LDL band was marked on the gel. The migration distance of the bands from the top of the gel was measured. The average LDL particle diameter was estimated from a quadratic extrapolation of a plot of the logarithm of the diameter of the standards versus the migration distance of the standards [39].

Autoantibodies against malondialdehyde-modified LDL

Malondialdehyde-modified LDL (MDA-LDL) was prepared as described by Palinski et al [40].

Concentrations of autoantibodies against MDA-modified LDL were determined by ELISA [41]. The method was modified after the solid-phase radioimmunoassay as described by Salonen et al [20]. Microtiter plates with high binding capacity (Greiner no. 655061) were coated with 50 μl MDA-LDL (LDL-cholesterol = 14 to 16 μM) or native LDL at the same concentration in phosphate buffered saline (PBS: 0.15 M NaCl, 0.05 M phosphate, pH 7.4) containing 0.27 mM EDTA and 20 μM butylated hydroxytoluene (BHT) for two hours at 37°C. After incubation, each plate was washed with PBS containing 0.05% Tween-20 and 0.001% aprotinin using a microplate washer (Biorad model 1550). The remaining binding sites were blocked with 150 μl 2% bovine serum albumin (BSA) in PBS for two hours at room temperature. The BSA-solution was heated at 56°C for 30 minutes, filtered over a paper filter and cooled to room temperature before use. Each plate was washed as described above. Duplicate plasma samples in dilution of 1/1667 for IgM class and 1/833.3 for IgG class, in a total volume of 50 μl were added to the wells and incubated overnight at 4°C. Wells not incubated with MDA-LDL were used as a blank. The next day the wells were aspirated and washed again as described above. Fifty microliters of a thousand-fold diluted monoclonal mouse antibody against human IgM or IgG (Sigma Immunochemicals, St. Louis, MO, USA) in PBS was added and the plates were incubated for four hours at 4°C and subsequently washed as described above. Fifty microliters of a peroxidase conjugated goat anti-mouse antibody (Tago Inc., Burlingname,

Table 2. Lipids and lipoproteins in renal transplant recipients and controls

Variable	Kidney recipients	Controls	*P* value
Total cholesterol	5.91 ± 0.95	5.33 ± 0.50	0.017
Total triglyceride	2.40 ± 0.99	1.43 ± 0.65	0.001
HDL-cholesterol	1.09 ± 0.39	1.21 ± 0.23	0.28
LDL-cholesterol	3.73 ± 0.70	3.44 ± 0.44	0.13

All variables are in mM ± standard deviation.

Table 3. Low density lipoprotein size and subclass patterns in kidney transplant patients and controls

Variable	Patients (19)	Controls (19)
LDL size (Å)	236.5 ± 7.3[a]	247.8 ± 11.6
LDL subclass		
Pattern A	6[b]	13
Pattern A/B	2	1
Pattern B	11[b]	5

Significantly different from controls ([a] Student's *t*-test, $P < 0.002$, [b] Chi-square test, $P = 0.028$)

Table 4. Lipids and lipoproteins in subjects with different LDL subclass patterns

Variable	Pattern A	Pattern A/B	Pattern B
Total triglyceride	1.39 ± 0.58	1.75 ± 0.21	2.60 ± 1.00[a]
Total cholesterol	5.40 ± 0.74	5.40 ± 0.40	5.91 ± 0.87
LDL-cholesterol	3.52 ± 0.53	3.51 ± 0.61	3.68 ± 0.69
HDL-cholesterol	1.24 ± 0.26	1.07 ± 0.08	1.06 ± 0.39

All variables are in mM.

[a] Statistically significant difference between LDL subclass pattern A and B, $P < 0.001$

CA, USA) were added to the wells in a thousand-fold dilution in PBS and incubated for one hour at 37°C, followed by the washing procedure. For the substrate reaction, an orthophenylenediamine dihydrochloride solution (2 mg/ml) (Sigma Chemical Co., St. Louis, MO, USA) in citric acid (0.1 M) phosphate (0.2 M) buffer (pH 5.5), containing 0.015% hydrogen peroxide was prepared just before use. Fifty microliters of the substrate solution were added to each well and incubated in the dark during exactly 20 minutes. The reaction was stopped with 50 µl 2.5 M H_2SO_4 per well.

Absorbance was measured using a microplate reader (Biorad model 450) at 490 nm. The absorbance was linear with the amount of diluted plasma added up to 100 µl. The antibody titer was defined as the absorbance of the wells coated with MDA-LDL divided by the absorbance of the wells coated with native LDL for each plasma sample [20]. The concentration of MDA specific autoantibodies was calculated from the difference in absorbance between the MDA-LDL coated wells and native LDL coated wells for each sample. The absorbances were converted in µg antibody/ml plasma by comparison with a standard curve of a pool plasma with known anti-MDA-LDL antibody concentrations (IgM 27.0 ± 2.0 µg/ml, IgG 5.8 ± 0.1 µg/ml) [41].

Other analytical methods

Plasma cholesterol and triglycerides (Boehringer Mannheim, Mannheim, Germany) and creatinine (Sigma Diagnostics, St. Louis, MO, USA) were determined using commercially available test kits. An estimate of the creatinine clearance rate was calculated from the plasma creatinine concentration as described by Cockcroft and Gault [42].

Statistical analysis

Data are presented as means ± SD. Differences between groups were evaluated for significance using the Student-*t*-test or by ANOVA followed by Bonferoni for comparison of groups. Simple correlations between variables were calculated using the Pearson correlation test. The level of significance was set at $P < 0.05$.

Results

Lipids and lipoproteins in renal transplant recipients and controls

Plasma triglyceride and cholesterol levels were significantly higher in renal transplant recipients than in controls (68% and 12%, respectively; Table 2). In 47% (9 of 19) of the patients, cholesterol was > 6.2 mM (240 mg/dl), while in the control group only one subject had a cholesterol of 6.2 mM. LDL cholesterol tended to be higher in the patients than in the controls (+ 8%, $P = 0.13$). In 37% (7 of 19) of the patients and in one control LDL cholesterol was > 4.0 mM (155 mg/dl). The mean HDL cholesterol in the patients was not different from controls.

LDL size and subclass pattern

The mean size of the most prominent LDL fraction was significantly less in the patients than in controls (Table 3). The size of LDL was inversely correlated with the plasma triglyceride ($r = -0.66$, $P < 0.001$) and weakly positively with HDL cholesterol ($r = 0.34$, $P < 0.05$). The smaller size of the LDL particles was reflected in the LDL subclass pattern. The LDL subclass pattern B, with a mean diameter of the major LDL subfraction of 233.3 ± 3.1 Å, was more frequently found in the patients (58%) than in the controls (29%)(Pearsons chi-square test, $P = 0.028$, Table 3). The LDL subclass pattern A (mean particle diameter 250.9 ± 9.2 Å) was present in 26% of the patients and in 68% of the controls. The mean size of the major LDL fraction in the LDL subclass pattern A and B in controls and patients were not significantly different (controls vs. patients, pattern A 253.4 ± 9.2 Å vs. 245.6 ± 6.1 Å, pattern B 235.9 ± 3.6 Å vs. 232.1 ± 2.2 Å). An intermediate LDL subclass pattern was found in two patients and in one control.

The LDL subclass pattern B was associated with a higher plasma triglyceride and a lower HDL cholesterol (Table 4). Considering a plasma triglyceride above 2.3 mM and HDL cholesterol beneath 0.9 mM as abnormal, in 7 patients small-dense LDL together with decreased HDL cholesterol and increased plasma triglyceride was found, in contrast to the control group in which only one subject met these criteria. LDL cholesterol was similar among subjects with different LDL subclasses. There was no relation between LDL size and LDL cholesterol or total plasma cholesterol (not shown).

Low density lipoprotein oxidation

The susceptibility of LDL to oxidation was determined by following *in vitro* Cu^{2+}-induced LDL oxidation. In the kidney recipients the lag time was 32% shorter than in controls (Table 5). In subjects with an LDL subclass pattern B the lag phase was significantly shorter than in subjects with an LDL subclass pattern A (−38%; Table 5). In patients and controls with the same

Table 5. Lag time of low density lipoprotein oxidation *in vitro*

LDL subclass	All	Kidney recipients	Controls
All	124 ± 63 (38)	101 ± 23 (19)	148 ± 81 (19)[c]
Pattern A	154 ± 78 (19)[a]	122 ± 30 (6)	169 ± 80 (13)
Pattern A/B	94 ± 10 (3)	94 ± 15 (2)	97 (1)
Pattern B	95 ± 15 (16)[b]	92 ± 11 (11)	102 ± 21 (5)

Given are the lag times in min. Between brackets the number of samples is shown.

[a] Significantly different from [b] (ANOVA followed by Bonferoni, $P < 0.02$

[c] Statistically significant difference between patients and controls, $P = 0.02$

Table 6. Autoantibodies against MDA-LDL in renal transplant patients and controls

Variable	Kidney recipients	Controls	*P* value
Antibody concentration			
IgM *μg/ml*	49.4 ± 15.9	24.4 ± 8.7	< 0.0001
IgG *μg/ml*	5.6 ± 3.8	3.2 ± 1.9	< 0.02
Antibody titer			
IgM	2.10 ± 0.32	1.56 ± 0.22	< 0.0001
IgG	1.25 ± 0.19	1.15 ± 0.10	< 0.05

subclass pattern, the lag time tended to be shorter in the patients but the differences were not statistically significant (Table 5). There was no correlation between plasma LDL cholesterol and lag time in neither controls nor patients (not shown).

Autoantibodies against MDA-modified LDL

Concentrations and titers of autoantibodies against MDA-LDL were significantly higher in renal transplant patients than in controls (Table 6). IgM autoantibody concentrations in the patients were on the average about twofold higher than in the controls. IgG autoantibodies were 75% higher in the renal transplant group. The titer of the IgM autoantibodies against MDA-LDL was 35% and of the IgG autoantibodies 9% higher in the patients than in the controls, both being statistically significantly different (Table 6). In patients with an LDL subclass pattern B IgM antibody values were higher than in subjects with the pattern A, IgM antibodies pattern B versus pattern A 48.3 ± 17.6 μg/ml versus 26.2 ± 10.0 μg/ml ($P < 0.001$), IgM antibody titers 2.11 ± 0.35 versus 1.58 ± 0.21 ($P < 0.001$). Pattern A and pattern B subjects did not differ in IgG autoantibodies. There was no correlation between LDL cholesterol and any of the antibody parameters nor between the plasma cyclosporine A content and any of the determined variables (not shown).

Discussion

In kidney transplant recipients varying degrees of hyperlipidemia have been reported [2, 43–48], with HDL cholesterol being lowered, enhanced or unaffected [10, 47, 48]. Our study group, which consisted of patients in a stable condition, resembled the average lipid profile in kidney transplant patients with an increased plasma cholesterol [>6.2 mM (240 mg/dl)] in 47% of the patients. Since plasma triglyceride was elevated part of the hypercholesterolemia was attributable to an increase in VLDL cholesterol. Still, 37% of the patients had an LDL cholesterol above 4 mM (155 mg/dl). Kidney transplantation is associated with an increased occurrence of atherosclerosis. Several factors are associated with the atherosclerosis, hypercholesterolemia being one of them [2]. Over the years it has become clear that in addition to an enhanced LDL cholesterol other lipoprotein-associated factors are related with the risk of atherosclerosis. Oxidation of LDL is considered to be the major event in the development of atherosclerosis. We found that parameters of *in vitro* (lag phase of LDL oxidation) and *in vivo* (autoantibodies against MDA-LDL) LDL oxidation are greatly affected in renal transplant patients, indicating that LDL oxidation may be increased in these patients. We, therefore, think that one cause of the aggravated atherosclerosis in renal transplant patients may be an increased LDL oxidation. Recently, Maggi and coworkers reported enhanced levels of autoantibodies against MDA-LDL in patients during chronic hemodialysis [49]. During this treatment, which often precedes transplantation, atherosclerosis is probably initiated. After transplantation, the atherosclerosis may progress even more. The rate of *in vivo* LDL oxidation is presumably dependent on several factors. Renal insufficiency does not seem to be a major determinant of the LDL oxidation as none of the oxidation parameters were correlated with creatinine clearance in patients or controls. The susceptibility of LDL for oxidation, which is reflected in the lag phase of copper-induced *in vitro* LDL oxidation, is probably an important factor determining the *in vivo* LDL oxidation rate. The LDL size is one determinant of its susceptibility for *in vitro* oxidation, small-dense LDL being more susceptible than larger LDL [32, 33]. This was also found in the patients and controls in the present study. Another factor influencing the LDL oxidation in transplant patients may be the use of cyclosporine A. Apanay and coworkers [11] found in renal transplant patients with a high plasma cyclosporine level shorter lag phases than in controls and in patients with lower cyclosporine levels. Moreover, an inverse correlation between the amount of LDL-associated cyclosporine and the lag phase was found to exist. We found no correlation between the lag phase and the plasma cyclosporine level, although the lag phases of the renal transplant recipients tended to be shorter if compared to controls with the same LDL subclass. Therefore, cyclosporine may have a small effect on the lag phase, but it seems that the difference in the mean LDL oxidation lag time between control and patient groups is mainly due to the high number of subjects with small LDL in the renal transplant group. Recently, we demonstrated that, in subjects with coronary artery disease, autoantibodies against MDA-LDL were higher in patients with the LDL subclass pattern B than with the LDL subclass pattern A [41]. In the renal transplant patients this was also the case. This suggests that not only *in vitro*, but also *in vivo*, LDL of patients with the LDL subclass pattern B is more readily oxidized than larger LDL. In this respect it is of interest that Taylor and coworkers [50] showed that the oxidation potential is higher in renal transplant recipients than in controls as evidenced by higher plasma malondialdehyde levels, lower plasma thiols and increased red cell superoxide dismutase. Thus several factors may add to an enhanced LDL oxidation in the patients: a larger number of patients with small-dense LDL, increased susceptibility for oxidation by cyclosporine and a higher overall oxidation potential. The presence of small-dense LDL may be at least in part metabolically explained. The LDL subclass pattern B is associated with an increased (VLDL) triglyceride and low HDL-C [38, 51]. In our study a lower LDL size was also associated

with higher plasma triglyceride and lower HDL cholesterol levels. If the lowering in LDL size is due to the increase in triglycerides, the increased plasma triglyceride level may be one cause of enhanced LDL oxidation. Supposing that increased LDL oxidation contributes to the high atherosclerotic disease in the transplant patients, a lowering of plasma triglycerides (if it leads to a shift of LDL subclass pattern and subsequently less LDL oxidation) may help to diminish the progression of atherosclerosis in renal transplant patients.

In conclusion, our results show that in renal transplant recipients, there is increased incidence of the LDL subclass pattern B with an increased susceptibility to oxidative modification. Probably LDL oxidation *in vivo* is also increased in transplant patients, as indicated by increased values of autoantibodies against MDA-LDL. These factors may play a role in the accelerated atherogenesis occurring after renal transplantation.

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Reprint requests to Prof. Hans Jansen, Ph.D., Department of Internal Medicine III, BD 277, Erasmus University Rotterdam, POB 1738, 3000 DR Rotterdam, The Netherlands. E-mail: jansen@bc1.fgg.eur.nl

References

1. LAZARUS JM, LOWRIE EG, HAMPERS CL, MERRIL JP: Cardiovascular disease in uremic patients on hemodialysis. *Kidney Int* 7:167–175, 1975
2. KASISKE BL: Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med* 84:985–992, 1988
3. LINDNER A, CHARRA B, SHERRARD DJ, SCRIBNER BH: Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 290:697–701, 1974
4. IBELS LS, STEWART JH, MAHONY JF, NEALE FC, SHEIL AGR: Occlusive arterial disease in uraemic and haemodialysed patients and renal transplant recipients. *Q J Med* 182:197–214, 1977
5. CURTIS JJ: Hypertension and kidney transplantation. *Am J Kidney Dis* 7:181–196, 1986
6. CHAPMAN JR, MARCEN R, ARIAZ M, RAINE AEG, DUNNILL MS, MORRIS PJ: Hypertension after renal transplantation. A comparison of cyclosporin and conventional immunosuppression. *Transplantation* 43:860–864, 1987
7. CATTRAN DC, STEINER G, WILSON DR, FENTON SSA: Hyperlipidemia after renal transplantation: Natural history and pathophysiology. *Ann Intern Med* 91:554–559, 1979
8. LOWRY RP, SOLTYS G, MANGEL R, KWITEROVITCH P, SNIDERMAN AD: Type II hyperlipoproteinemia, hyperapobetalipoproteinemia, and hyperalphalipoproteinemia following renal transplantation: Prevalence and precipitating factors. *Transplant Proc* 19:2229–2232, 1987
9. KASISKE BL, UMEN AJ: Persistent hyperlipidaemia in renal transplant patients. *Medicine* 66:309–316, 1987
10. TELCI A, SALMAYENLI N, AYDIN AE, YAMANER S, SIVAS A, ELDEGEZ U: Serum lipids and apolipoprotein concentrations and plasma fibronectin concentrations in renal transplant patients. *Eur J Clin Chem Clin Biochem* 30:847–850, 1992
11. APANAY DC, NEYLAN JF, RAGAB MS, SGOUTAS DS: Cyclosporin increases the oxidizability of low-density lipoproteins in renal transplant recipients. *Transplantation* 58:663–669, 1994
12. STEINBERG D, PARTHASARATHY S, CAREW TE, KHOO JC, WITZTUM JL: Beyond cholesterol: Modifications of LDL that increase its atherogenicity. *N Engl J Med* 320:916–924, 1989
13. HENRIKSEN T, MAHONEY EM, STEINBERG D: Enhanced macrophage degradation of biologically modified low density lipoproteins. *Arteriosclerosis* 3:149–159, 1983
14. HABERLAND ME, FONG D, CHENG L: Malondialdehyde-altered protein occurs in atheroma of Watanabe heritable hyperlipidemic rabbits. *Science* 241:215–218, 1988
15. YLÄ-HERTTUALA S, PALINSKI W, ROSENFELD ME, PATHASARATHY S, CAREW TC, BUTLER S, WITZTUM JL, STEINBERG D: Evidence for the presence of oxidatively modified LDL in atherosclerotic lesions of rabbit and man. *J Clin Invest* 84:1086–1095, 1989
16. BOYD HC, GOWN AM, WOLFBAUER G, CHAIT A: Direct evidence for a protein recognized by a monoclonal antibody against oxidatively-modified LDL in atherosclerotic lesions from a Watanabe heritable hyperlipidemic rabbit. *Am J Pathol* 135:815–825, 1989
17. ESTERBAUER H, STRIEGL G, PUHL H, ROTHENEDER M: Continuous monitoring of in vitro oxidation of human LDL. *Free Radical Res Commun* 6:67–75, 1989
18. REGNSTROM J, NILSSON J, TORNVALL P, LANDOU C, HAMSTEN A: Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. *Lancet* 339:1183–1186, 1992
19. CHAIT A: Methods for assessing lipid and lipoprotein oxidation. *Curr Opin Lipid* 3:389–394, 1992
20. SALONEN JT, YLÄ-HERTTUALA S, YAMAMOTO R, BUTLER S, KORPELA H, SALONEN R, NYYSSÖNEN K, PALINSKI W, WITZTUM JL: Autoantibody against oxidized LDL and progression of carotid atherosclerosis. *Lancet* 339:883–887, 1992
21. SHEN MM, KRAUSS RM, LINDGREN FT, FORTE TM: Heterogeneity of serum low density lipoproteins in normal human subjects. *J Lipid Res* 22:236–244, 1981
22. KRAUSS RM, BURKE DJ: Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J Lipid Res* 23:97–104, 1982
23. PACKARD CJ, SHEPHERD J, JOERNS S, GOTTO AM, TAUNTON OD: Very low density and low density lipoprotein subfractions in type III and type IV hyperlipoproteinemia. *Biochim Biophys Acta* 572:269–282, 1979
24. RUBENSTEIN B, STEINER G: Fractionation of human low density lipoprotein by column chromatography. *Can J Biochem* 22:1023–1028, 1976
25. AUSTIN MA, KRAUSS RM: Genetic control of low density lipoprotein subclasses. *Lancet* 2:592–595, 1986
26. AUSTIN MA, BRESLOW JL, HENNEKENS CH, BURING JE, WILLETT WC, KRAUSS RM: Low density lipoprotein subclass patterns and the risk of myocardial infarction. *JAMA* 260:1917–1921, 1988
27. KRAUSS RM: Relationship of intermediate and low density lipoprotein subspecies to risk of coronary artery disease. *Am Heart J* 113:578–582, 1987
28. CROUSE JR, PARKS JS, SCHEY HM, KAHL FR: Studies of low density lipoprotein molecular weight in human beings with coronary artery disease. *J Lipid Res* 26:566–574, 1985
29. CAMPOS H, GENEST JR JJ, BLIJLEVENS E, MCNAMARA J, JENNER JL, ORDOVAS JM, WILSON PWF, SCHAEFER EJ: Low density particle size and coronary artery disease. *Arterioscler Thromb* 12:187–195, 1992
30. LABELLE M, KRAUSS RM: Differences in carbohydrate content of low density lipoproteins associated with low density lipoprotein subclass patterns. *J Lipid Res* 31:1577–1588, 1990
31. AVILA EM, LOPEZ F, CAMEJO G: Properties of low density lipoprotein related to its interaction with arterial wall components. In vitro and vivo studies. *Artery* 4:36–60, 1978
32. DEGRAAF J, HAK-LEMMERS HL, HECTORS MC, DEMACKER PN, HENDRIKS JC, STALENHOEF AFH: Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arterioscl Thromb* 11:298–306, 1991
33. CHAIT A, BRAZG RL, TRIBBLE DL, KRAUSS RM: Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, Pattern B. *Am J Med* 94:350–356, 1993
34. KLEINVELD HA, HAK-LEMMERS HLM, STALENHOEF AFH, DEMACKER PNM: Improved measurement of low-density lipoprotein susceptibility to copper-induced oxidation: Application of a short procedure for isolating low-density lipoprotein. *Clin Chem* 38:2066–2072, 1992
35. REDGRAVE TG, ROBERTS DCK, WEST E: Separation of plasma lipoproteins by density gradient ultracentrifugation. *Anal Biochem* 65:42–49, 1975
36. BURSTEIN M, SCHOLNICK HR, MORFEN R: Rapid method for the

isolation of lipoproteins from human serum by precipitation with polyanions. *J Lipid Res* 11:583–595, 1970
37. FRIEDEWALD WT, LEVY RI, FREDRICKSON DS: Estimation of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
38. JANSEN H, HOP W, VAN TOL A, BRUSCHKE AVG, BIRKENHÄGER JC: Hepatic lipase and lipoprotein lipase are not major determinants of low density lipoprotein subclass pattern in human subjects with coronary heart disease. *Atherosclerosis* 107:45–54, 1994
39. CORESH J, KWITEROVICH PO, SMITH HH, BACHORIK PS: Association of plasma triglyceride concentration and LDL particle diameter, density, and chemical composition with premature coronary artery disease in men and women. *J Lipid Res* 34:1687–1697, 1993
40. PALINSKI W, YLÄ-HERTTUALA S, ROSENFELD ME, BUTLER SW, SOCHER SA, PARTHASARATHY S, CURTISS LK, WITZTUM JL: Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 10:325–335, 1990
41. JANSEN H, GHANEM H, KUYPERS HSAM, BIRKENHÄGER JC: Autoantibodies against malondialdehyde-modified LDL are enhanced in subjects with an LDL subclass B pattern. *Atherosclerosis* 115:255–262, 1995
42. COCKCROFT DW, GAULT MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
43. MOORE R, THOMAS D, MORGAN E, WHEELER D, GRIFFIN P, SALAMAN J, REES A: Abnormal lipid and lipoprotein profiles following renal transplantation. *Transplant Proc* 25:1060–1061, 1993
44. ALLEGRA V, MENGOZZI G, VASILE A: Lipoprotein lipids and apoproteins in healthy renal transplant recipients. *Nephron* 51:276–277, 1989
45. IBELS LS, REARDON MF, NESTEL PJ: Plasma post-heparin lipolytic activity and triglyceride clearance in uraemic and hemodialysis patients and renal allograft recipients. *J Lab Clin Med* 87:648–658, 1976
46. KOBAYASHI N, OKUBO M, MARUMO F, UCHIDA H, ENDO T, NAKAMURA H: De novo development of hypercholesterolemia and elevated high density lipoprotein cholesterol: Apoprotein AI ratio in patients with chronic renal failure following kidney transplantation. *Nephron* 35:237–240, 1983
47. IBELS LS, SIMONS LA, KING JO, WILLIAMS PF, NEALE FC, STEWART JH: Studies on the nature and causes of hyperlipemia of uremia maintenance dialysis and renal transplantation. *Q J Med* 44:601–614, 1975
48. BAGDADE J, CASARETTO A, ALBERTS J: Effects of chronic uremia, hemodialysis and renal transplantation on plasma lipids and lipoproteins in man. *J Lab Clin Med* 87:38–48, 1976
49. MAGGI E, BELLAZZI R, GAZO A, SECCIA M, BELLOMO G: Autoantibodies against oxidatively-modified LDL in uraemic patients undergoing dialysis. *Kidney Int* 46:869–876, 1994
50. TAYLOR JE, SCOTT N, HILL A, BRIDGES A, HENDERSON IS, STEWART WK, BELCH JJF: Oxygen free radicals and platelet and granulocyte aggregability in renal transplant patients. *Transplantation* 55:500–504, 1993
51. AUSTIN MA, BRUNZELL JD, FITCH WL, KRAUSS RM: Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis* 10:520–530, 1990